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DATA EVALUATION RECORD

I. SUMMARY

MRID (Acc.) No.: 412552-25
ID No.: 7078-RT
RD Record No.: 253,112
Caswell No.: 623C (129017)
Project No.: 0-0339

Study Type: Mutagenicity - Chromosome damage in vitro (CA/CHO)

Chemical: CIDEX OPA Antimicrobial (ortho-phthalaldehyde)

Sponsor: Surgikos, Inc., Arlington, TX

Testing Facility: Microbiological Associates (M/A)
Bethesda, MD

Title of Report: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells.

Authors: D.L. Putnam and M.M. Morris

Study Number: (M/A) T8241.337

Date of Issue: December 28, 1988

TB Conclusions:

Positive for increased chromosome damage in a dose-responsive manner in both activated and nonactivated CHO cells exposed at levels of 0.7 to 5.0 ug/mL.

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - 913-12 (o-phthalaldehyde, OPA)

Description: Light yellow crystalline solid
Batch (Lot): 861-65
Purity (%): 99
Solvent/Carrier/Diluent: Distilled water (DW)

B. Test Organisms - Mammalian cell strain

Species: Chinese hamster (ovary, CHO)
Strain: K1
Source: American Type Culture Collection (CCL#61), Rockville, MD

C. Study Design (Protocol) - This study was designed to assess the clastogenic (chromosome-breaking) potential of OPA when administered in vitro to CHO cells according to an enclosed protocol based upon recognized (published) procedures and methods.

Statements of Quality Assurance measures (inspections/audits) as well as adherence to Good Laboratory Practice were provided.

D. Procedures/Methods of Analysis - Following preliminary cytotoxicity testing to select dosages for the main assay (nine concentrations of OPA ranging from 0.05 to 500 ug/mL), duplicate cultures of CHO cells were exposed to graded concentrations of test article for 16 hours under nonactivation conditions, but only for 2 hours in the presence of mammalian metabolic activation* (followed by 14 hours further incubation in nontest fresh culture medium). Two hours before harvest, the mitotic poison Colcemid (0.1 ug/mL) was added to collect cells in metaphase. In addition to solvent (DW) controls, other cultures were treated with the clastogens triethylenemelamine (TEM, 0.5 ug/mL) and cyclophosphamide (CP, 50 ug/mL) to serve as positive controls for the nonactivated and S9-supplemented test series, respectively.

At harvest (18 hours after treatment initiation), all cultures were centrifuged and microscopic slide preparations made according to conventional cytological methods. Giemsa-stained coded preparations (50 metaphases per flask, 100 per treatment) were scored for the conventional

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*Microsomal enzymes (S9) liver homogenates from male S-D rats pretreated with Aroclor 1254, plus cofactors.

array of structural chromatid and chromosome aberrations (simple breaks and acentrics; interchanges and other complex rearrangements; dicentrics, rings, and fragments; pulverization and severely damaged cells with > 10 aberrations). In addition, mitotic indices (MI) were recorded for all cultures (= percent cells in mitosis per 500 cells counted).

Chromosome data were analyzed by Fisher's Exact Test for pairwise comparisons between test groups and solvent control. In the event of a positive Fisher's ($p < 0.05$ or $p < 0.01$), the Cochran-Armitage procedure was applied to determine dose-responsiveness.

For an assay to be analyzed, this lab requires that the negative (solvent) control value be no more than 6 percent aberrations, but that the positive control(s) must be statistically increased over solvent values.

E. Results - Based upon severe growth inhibition (significantly reduced MI and cell cycle delay) above 5 ug/mL OPA in preliminary toxicity testing, 5 ug/mL was selected as the highest dose to be tested (HDT) in the aberration assay for both the activated and nonactivated series (Report Tables 1 and 2). In addition, four lower concentrations were chosen: 0.35, 0.70, 1.30, and 2.50 ug/mL.

In the single complete aberration assay conducted, dose-related increased chromosome damage over solvent controls (essentially 0% aberrations) was recorded, beginning at the mild to moderately toxic dose of 1.3 ug/mL under both activation and nonactivation conditions, becoming significant (Fisher's Exact Test) at 2.5 ug/mL ($p < 0.05$) and 5.0 ug/mL ($p < 0.01$) without S9, but only at 5.0 ug/mL ($p < 0.01$) with S9 (Report Tables 3, 4, and 5 attached to this DER). Both positive controls responded appropriately, with large increases ($p < 0.01$) in chromosome damage (5 to 10X background).

The authors concluded that the test article, OPA technical, was positive in this test system.

F. TB Evaluation - ACCEPTABLE. Although only a single trial was conducted, this cytogenetic study was carried out with adequate procedures and appropriate controls such that it was amply demonstrated that the test substance was clastogenic in vitro in CHO cells.

Attachments (Data Tables)

ATTACHMENT I

Data Tables

Page _____ is not included in this copy.

Pages 5 through 7 are not included in this copy.

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